We claim:

1. An aqueous composition buffered to a pH of from about 7 to about 9, and comprising:

a) first and second primers which are specific to and hybridizable with, respectively, first and second nucleic acid sequences which are in opposing strands of a first target DNA and which are separated from each other along said opposing strands by from 90 to 400 nucleotides, and

b) third and fourth primers which are specific to and hybridizable with, respectively, third and fourth nucleic acid sequences which are in opposing strands of a second target DNA which is the same as or different from said first target DNA, said third and fourth nucleic acid sequences being different from said first and second nucleic acid sequences and being separated from each other along said opposing strands by from 90 to 400 nucleotides,

each of said first, second, third and fourth primers having a T_m within the range of from about 65 to about 74°C, all of said primer T_m's being within about 5°C of each other, said first and second primers having nucleotide lengths which differ from each other by no more than 5 nucleotides, and said third and fourth primers having nucleotide lengths which differ from each other by no more than 5 nucleotides.

2. The composition of claim 1 wherein said first and second primers are specific to and hybridizable with first and second nucleic acid sequences which are in opposing strands of a DNA associated with a first infectious agent, and said third and fourth primers are specific to and hybridizable with third and fourth nucleic acid sequences which are in opposing strands of a DNA associated with a second infectious agent.

3. The composition of claim 1 wherein each of said primers is present at a concentration of at least about 0.075 µmolar, and said composition further comprises

a thermostable DNA polymerase present at from about 0.1 to about 50 units/100 µl,

a DNA polymerase cofactor present at from about 2 to about 15 mmolar, and

a dNTP present at from about 0.25 to about 3.5 mmolar.

4. The composition of claim 1 wherein each of said first, second, third and fourth primers has from 20 to 40 nucleotides, and a T_m within the range of from about 67 to about 74°C, said primer T_m's being within about 2°C of each other.

5. The composition of claim 1 wherein said T_m values are calculated using the formula:

$$T_m\ (°C) = 67.5 + 0.34(\%G + C) - 395/N$$

wherein G and C represent the number of guanine and cytosine nucleotides, respectively, and N represents the total number of nucleotides.

6. The composition of claim 1 wherein either said first and second primers, or said third and fourth primers are specific to and hybridizable with said nucleic acid sequences which are in opposing strands of a DNA selected from the group consisting of a retroviral DNA, hCMV DNA, *Mycobacterium tuberculosis* DNA, human papilloma viral DNA, *Mycobacterium avium* DNA, hepatitis viral DNA and *Pneumocystis carinii* DNA.

7. The composition of claim 1 wherein one or both of said first and second primers, and one or both of said third and fourth primers, are labeled with the same or different specific binding moiety.

8. The composition of claim 7 wherein said labeled primers are labeled with biotin.

9. A diagnostic test kit for the amplification of first and second target DNA's comprising, in separate packaging:

a) an aqueous composition buffered to a pH of from about 7 to about 9, and comprising:

first and second primers which are specific to and hybridizable with, respectively, first and second nucleic acid sequences which are in opposing strands of a first target DNA and which are separated from each other along said opposing strands by from 90 to 400 nucleotides, and

third and fourth primers which are specific to and hybridizable with, respectively, third and fourth nucleic acid sequences which are in opposing strands of a second target DNA which is the same as or different from said first target DNA, said third and fourth nucleic acid sequences being different from said first and second nucleic acid sequences and being separated from each other along said opposing strands of said second target DNA by from 90 to 400 nucleotides,

each of said first, second, third and fourth primers having a T_m within the range of from about 65 to about 74°C, all of said primer T_m's being within about 5°C of each other, said first and second primers having nucleotide lengths which differ from each other by no more than 5 nucleotides, and said third and fourth primers having nucleotide lengths which differ from each other by no more than 5 nucleotides, and

b) at least one additional PCR reagent.

10. The test kit of claim 9 wherein said additional PCR reagent is a thermostable DNA polymerase, a DNA polymerase cofactor or a dNTP.

11. The test kit of claim 9 further comprising

a first capture reagent comprising a water-insoluble support to which is covalently attached a first capture probe which is specific to a nucleic acid sequence of a strand of said first target DNA, said capture probe having from 10 to 40 nucleotides and a T_m greater than about 50°C, and being hybridizable with said nucleic acid sequence of said first target DNA strand at a temperature in the range of from about 40 to about 55°C, and

a second capture reagent comprising a water-insoluble support to which is covalently attached a second capture probe which is specific to a nucleic acid sequence of a strand of said second target DNA, said second capture probe having from 10 to 40 nucleotides and a T_m greater than about 50°C, and being hybridizable with said nucleic acid sequence of said second target DNA strand at a temperature in the range of from about 40 to about 55°C.

12. The test kit of claim 9 wherein each of said primers is present at a concentration of at least about 0.075 µmolar, and said composition further comprises

a thermostable DNA polymerase present at from about 0.1 to about 50 units/100 µl,

a DNA polymerase cofactor present at from about 2 to about 15 mmolar, or

a dNTP present at from about 0.25 to about 3.5 mmolar.

13. The test kit of claim 9 wherein one or both of said first and second primers, and one or both of said third and fourth primers, are labeled with biotin, and said test kit further includes a conjugate of avidin with an enzyme and a substrate reagent which

provides a detectable signal in the presence of said enzyme.

14. The test kit of claim 13 wherein said conjugate comprises avidin and peroxidase, and said substrate reagent provides a detectable colorimetric or chemiluminescent signal in the presence of peroxidase and an oxidant.

15. A method for the simultaneous amplification and detection of a first target DNA and a second target DNA comprising:

A) simultaneously subjecting the denatured opposing strands of a first target DNA and the denatured opposing strands of a second target DNA to polymerase chain reaction in the presence of:

i) an aqueous composition buffered to a pH of from about 7 to about 9, and comprising:

first and second primers which are specific to and hybridizable with, respectively, first and second nucleic acid sequences which are in opposing strands of a first target DNA and which are separated from each other along said opposing strands by from 90 to 400 nucleotides,

third and fourth primers which are specific to and hybridizable with, respectively, third and fourth nucleic acid sequences which are in opposing strands of a second target DNA which is the same as or different from said first target DNA, said third and fourth nucleic acid sequences being different from said first and second nucleic acid sequences and being separated from each other along said opposing strands of said second target DNA by from 90 to 400 nucleotides,

each of said first, second, third and fourth primers having a T_m within the range of from about 65 to about 74°C, all of said primer T_m's being

within about 5°C of each other, said first and second primers having nucleotide lengths which differ from each other by no more than 5 nucleotides, and said third and fourth primers having nucleotide lengths which differ from each other by no more than 5 nucleotides, and

ii) the additional PCR reagents: a thermostable DNA polymerase, a DNA polymerase cofactor and dNTP's, any or all of said additional PCR reagents being in the same or a different composition as defined in i),

to simultaneously amplify said opposing first target DNA strands and said opposing second target DNA strands,

B) simultaneously detecting at least one of said amplified first target DNA strands and at least one of said amplified second target DNA strands as a simultaneous determination of the presence of said first and second target DNA's.

16. The method of claim 15 wherein each of said first, second, third and fourth primers has from 20 to 40 nucleotides, and a T_m within the range of from about 67 to about 74°C, said primer T_m's being within about 2°C of each other.

17. The method of claim 15 wherein one or both of said first and second primers, and one or both of said third and fourth primers, are labeled with the same or different specific binding moiety.

18. The method of claim 17 wherein said labeled primers are labeled with biotin, and detection of the resulting biotinylated amplified DNA strands for either target DNA is achieved by reacting said biotinylated amplified DNA strands with an avidin-enzyme conjugate, followed by reaction of said enzyme

with a substrate reagent to produce a detectable colorimetric or chemiluminescent signal.

19. The method of claim 18 wherein said one or more biotinylated amplified target DNA strands are detected by contacting them with an avidin-peroxidase conjugate, followed by reaction of peroxidase, in the presence of an oxidant, with either: luminol to produce a detectable chemiluminescent signal, or a leuco dye to produce a detectable colorimetric signal.

20. The method of claim 15 wherein PCR is carried out for from 20 to 50 cycles.

21. The method of claim 20 wherein, in each PCR cycle, priming and primer extension are carried out at the same temperature within the range of from about 62 to about 75°C.

22. The method of claim 15 wherein one of said amplified first target DNA strands is captured with a first capture reagent comprising a water-insoluble support to which is covalently attached a first capture probe which is specific to a nucleic acid sequence of said first target DNA strand, said first capture probe having from 10 to 40 nucleotides and a T_m greater than about 50°C, and is hybridizable with said nucleic acid sequence of said first target DNA strand at a temperature in the range of from about 40 to about 55°C, and

one of said amplified second target DNA strands is captured with a second capture reagent comprising a second capture probe specific to a nucleic acid sequence of said second target DNA strand, said second capture probe having from 10 to 40 nucleotides and a T_m greater than about 50°C, and is hybridizable with said nucleic acid sequence of said second target DNA strand at a temperature in the range of from about 40 to about 55°C,

said first and second capture probes having T_m's which differ by no more than about 15°C.

23. The method of claim 22 wherein said water-insoluble support for each capture reagent is a polymeric or magnetic particle having a diameter in the range of from about 0.001 to 10 micrometers, and each of said capture probes has a T_m greater than about 55°C.

24. The method of claim 22 wherein said first and second capture reagents ~~are~~ disposed in distinct regions on a water-insoluble substrate of a test device.

25. The method of claim 15 wherein said first and second target DNA's are associated with the same or different infectious agent.

26. The method of claim 25 wherein said first and second target DNA's are selected from the group consisting of a retroviral DNA, hCMV DNA, *Mycobacterium tuberculosis* DNA, human papilloma viral DNA, *Mycobacterium avium* DNA, hepatitis DNA and *Pneumocystis carinii* DNA.

27. The method of claim 15 wherein each of said primers is present at a concentration of at least about 0.075 µmolar,

a thermostable DNA polymerase is present at from about 0.1 to about 50 units/100 µl,

a DNA polymerase cofactor is present at from about 2 to about 15 mmolar, and

a dNTP is present at from about 0.25 to about 3.5 mmolar.

28. The method of claim 15 wherein three or more target DNA's are amplified using a set of primers for each of said target DNA's, the primers in each of said primer sets having a T_m within the range of from about 65 to about 74°C, all of said primer T_m's being

within about 5°C of each other, and said primers in each primer set having nucleotide lengths which differ from each other by no more than 5 nucleotides.

29. The method of claim 28 wherein each of said amplified target DNA's is captured with a capture reagent comprising a water-insoluble support to which is covalently attached a capture probe which is specific to a nucleic acid sequence of a strand of a distinct amplified target DNA strand, each capture probe having from 10 to 40 nucleotides and a T_m greater than about 50°C, and being hybridizable with said nucleic acid sequence of said distinct amplified target DNA strand at a temperature in the range of from about 40 to about 55°C.

30. A diagnostic element comprising a water-insoluble, heat or ultrasonic sealable support, having disposed thereon in distinct regions thereof, a plurality of capture reagents,

each of said capture reagents having a capture probe specific for and hybridizable with a distinct target DNA associated with an infectious agent at a temperature of from about 40 to about 55°C, each of said capture probes having from 10 to 40 nucleotides and a T_m greater than about 50°C, and the T_m's of all capture probes differing by no more than about 15°C.

31. A method for preparing a reaction mixture for polymerase chain reaction of two or more target DNA's comprising:

A) choosing a set of primers for each distinct target DNA, the primers in each set chosen to be specific to and hybridizable with nucleic acid sequences which are in opposing strands of said distinct target DNA and which are separated from each other along said opposing strands of said distinct target DNA by from 90 to 400 nucleotides

each of said primers in each primer set having a T_m within the range of from about 65 to about 74°C, all of the primer T_m's being within about 5°C of each other, and said primers in each set having nucleotide lengths which differ from each other by no more than 5 nucleotides,

the T_m's being calculated using the formula:

$$T_m\ (°C) = 67.5 + 0.34(\%G + C) - 395/N$$

wherein G and C represent the number of guanine and cytosine nucleotides, respectively, and N represents the total number of nucleotides, and

B) mixing said sets of primers chosen in step A) with:

a thermostable DNA polymerase in an amount of from about 0.1 to about 50 units/100 µl,

a DNA polymerase cofactor in an amount of from about 2 to about 15 mmolar, and

each of dATP, dCTP, dGTP and dTTP being present in an amount of from about 0.25 to about 3.5 mmolar,

wherein each of said primers is present in the mixture at a concentration of at least about 0.075 µmolar.

add C1

32. An oligonucleotide which is

GAGATGGGAA TCCATATGCT GTATGTGAT,

GGACACAGTG GCTTTTGACA GTTAATACA,

GATGGTCCAG CTGGACAAGC AGAAC,

CCTAGTGTGC CCATTAACAG GTCTTC,

GACACAGAAA ATGCTAGTGC TTATGCAGC,

GGTGGACAAT CACCTGGATT TACTGCAAC,

CCTGATCTGT GCACGGAACT GAACACT,

CCCAGTGTTA GTTAGTTTTT CCAATGTGTC,

TGCCTGCGGT GCCAGAAACC GTTGAAT,

TGCTCGGTTG CAGCACGAAT GGCACT,

GAGCCGAACC ACAACGTCAC ACAATGTT,

GGACACACAA AGGACAGGGT GTTCAGAAA,

GCGACTCAGA GGAAGAAAAC GATG,

GAGATCGAGC TGGAGGATCC GTACG,

AGCTGCAGCC CAAAGGTGTT GGACT,

GGAACAACAT TAGAACAGCA ATACAACAAA CCG,

AATATTGTAA CCTTTTGTTG CAAGTGTGAC TC,

CCTATAGGTG GTTTGCAACC AATTAAACAC,

GAGGTATTTG AATTTGCATT TAAAGATTTA TTTGT,

GCAAGACAGT ATTGGAACTT ACAGAGG,

GTGTTGTAAG TGTGAAGCCA GATTTGA,

GAGCAGATTG CGGCCACCGC AGCGATTTCG,

CCGGGAGATG GGGGAGGCTA ACTGA,

GGGGTGGGGA AAAGGAAGAA ACGCG, or

AAAGACAGAA TAAAACGCAC GGGTGTTGGG TCG.